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FINAL REPORT

To the Office of Naval Research under Contract Nonr-839(31)  
with the Polytechnic Institute of Brooklyn

SYNTHETIC POLYMER STUDIES RELATING

TO ENZYMOLOGY

J. Shafer and H. Morawetz,  
(Project Supervisor)

Nov. 1, 1960-Jan. 31, 1962

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INDEXSummaryResults and Discussions

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- B. The carboxylate catalyzed hydrolysis of m-acetoxy-trimethylanilinium iodide.
- C. Hydrolysis of an ester with a neighboring carboxyl and a quaternary ammonium group.
- D. The effect of a neighboring amide group on the hydrolysis of esters and amides.
- E. Hydrolysis of an amide with two neighboring carboxyl groups.
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### SUMMARY

A. An attempt was made to synthesize a polymer with a specific affinity for cholinesterase by attaching m-hydroxy trimethylanilinium functions to the polymer backbone. The synthesis proved more difficult than anticipated and this part of the project was abandoned.

B. The hydrolysis of m-acetoxytrimethylanilinium iodide catalyzed by singly, doubly and triply ionized citric acid showed the doubly ionized citrate to have an anomalously low catalytic activity. The possible reasons of this phenomenon are discussed.

C. The hydrolysis rate of B-N,N-dimethylamino<sup>ethyl</sup>hydrogen phthalate was studied over a range of pH values. The uncatalyzed rate is so high that it overshadows any possible contribution from the activation of the ester by an ionized neighboring carboxyl.

D. The base catalyzed hydrolysis rate of phthalamide and methyl phthalamate is very high due to the attack of the conjugate base of the amide on the amide or ester function. Phthalimide is a reaction intermediate in both cases.

E. The hydrolysis rate of N-(o-carboxy) phenylphthalamide acid was studied over a range of pH values. The rate is a maximum when one of the two carboxyl groups is ionized and it is then eighty times as fast as for the unionized amide. The effect is believed

to be due to a cooperative attack of an ionized and an unionized neighboring carboxyl on the amide function.

F. The hydrolysis of a compound carrying two cationic groups and two ester groups was studied in buffers and in partially ionized polymeric acids. The polyanions were found to act as powerful inhibitors for the hydroxyl ion catalyzed reaction. The data could be interpreted in terms of a polymer-ester association constant or in terms of the distribution of electrostatic potential in polyelectrolyte solution.

## RESULTS AND DISCUSSION

### A. Attempted Preparation of a Polymer with a Specific Affinity For Cholinesterase

#### I. Adsorption of Acetylcholinesterase on a commercial cationic resin

The purpose of this work was to investigate the possibility of separating an enzyme from a mixture of proteins by using polymers containing side groups which are specific inhibitors of the enzyme. Since the accepted mechanism of inhibition involves initial complex formation between inhibitor and enzyme, the enzyme was expected to be preferentially adsorbed on polymer containing inhibitor side groups. The polymer could be used as an adsorbent in chromatography, or as a coprecipitating agent in solution. A polymer containing carboxyl and inhibitor side groups could be precipitated from a solution containing an enzyme and protein mixture with barium salts. In such a case one would expect the enzyme to be coprecipitated with the polymer.

Work was started on this project using acetylcholinesterase. An enzyme-rich extract was obtained from Professor Wilson's Laboratory at Columbia University.

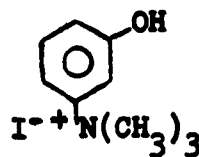
Since acetylcholinesterase is known to be inhibited by quaternary ammonium salts, the adsorption of the enzyme on resin IRA 401 (quaternary ammonium chloride type resin) was investigated. It was found that an enzyme solution in buffer at pH 7.2-7.4 which

stood over the resin lost all its activity (enzyme assayed by method of Histrin<sup>1</sup>) after three days. A blank enzyme solution containing no resin when kept under the same conditions did not lose activity. The loss in activity is attributed to adsorption of the enzyme on the resin. To evaluate the specificity of the resin for the enzyme, the adsorption of ox serum albumin was tested. It was found that 60% of the serum albumin was adsorbed from a solution standing over excess resin for 3 days.

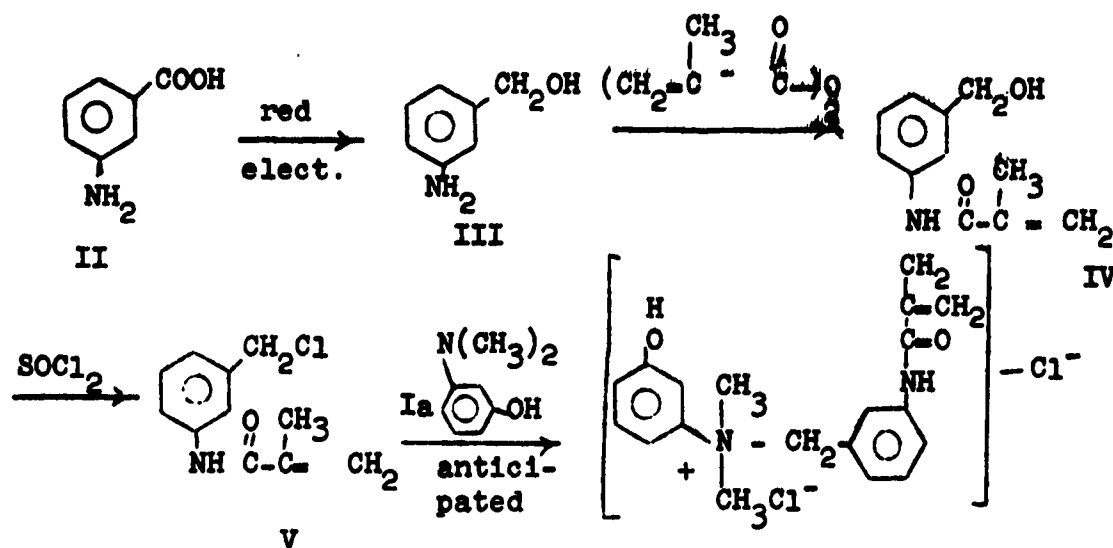
These results suggest that quaternary ammonium chloride ion exchange resins have a limited specificity for acetylcholinesterase, and may be used in the purification of the enzyme.

## II. Attempted Preparation of Monomers Containing Inhibitor Residues

Since 3-hydroxytrimethylanilium iodide (I) was reported to be 120 times more powerful an inhibitor for acetylcholinesterase than trimethyl ammonium iodide<sup>2</sup>, an attempt was made to synthesize a monomer with a 3-hydroxytrimethylanilium iodide residue. The following series of reactions were used.

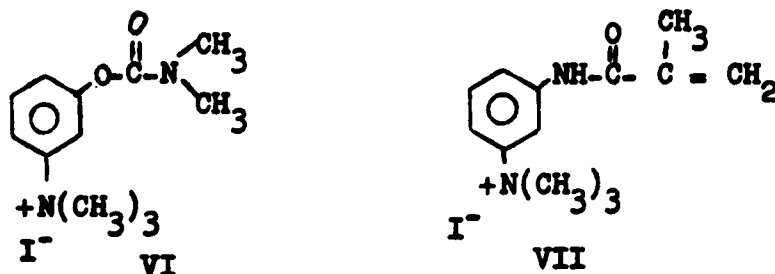


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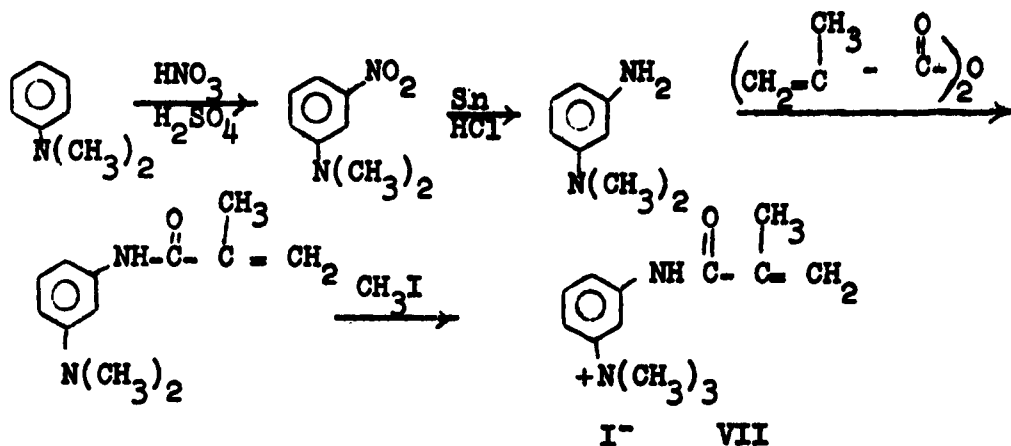


Compounds II-V were prepared and tentatively identified by their infrared spectra. However, all attempts to quaternize V with Ia led to a gummy resinous product.

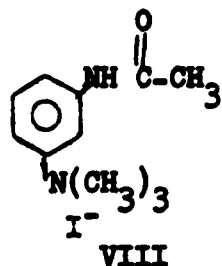
The very powerful inhibiting powers of the dimethylcarbamate of I, VI, has been ascribed to a critical distance between the cationic nitrogen and the carbonyl oxygen.<sup>2</sup> It was therefore thought that the 3-methacrylamidotrimethylanilium iodide (VII) with a very similar distance between carbonyl oxygen and positive nitrogen would be a good inhibitor.



Compound VII was made by the following reaction scheme and tentatively identified by its infrared spectrum



The method of Histrin was then used to ascertain its relative strength as an inhibitor of acetylcholinesterase. It was found to be much weaker than compound I or VI and was found to be comparable in strength to trimethylanilium chloride. The methacrylamido group in VII was replaced with an acetamido group VIII to ascertain if the relatively weak inhibitory power of VII was due to unsaturation.



compound VIII had the same activity as an inhibitor of acetylcholinesterase as VII. It was therefore concluded that other factors besides the distance

between carbonyl oxygen and cationic nitrogen are of major importance in determining the inhibitory powers of compounds for acetylcholinesterase action.

### Experimental

m-Aminobenzyl alcohol was prepared by electrolytically reducing m-aminobenzoic acid according to a method described by G. H. Coleman and H. L. Johnson, Organic Synthesis, Coll. Vol. III, p. 60-62. M.P. 92°C, lit. M.P. 92°C<sup>3</sup>.

Methacrylic Anhydride was prepared according to the method of R. K. Kulkarni, Dissertation for Ph.D., Polytechnic Institute of Brooklyn (1960).

#### m-Methacrylamidobenzyl Alcohol

To a 100 ml. roundbottom flask containing 7.38 g. (0.06 moles) of m-aminobenzylalcohol 9.24 g. (0.06 moles) of methacrylic anhydride containing hydroquinone is added all at once. The flask is kept in an ice water bath. The oil formed is stirred with a glass rod until it becomes a thick syrup. Then 60 cm<sup>3</sup> of water is added and an efficient mechanical stirrer is attached to the flask. The mixture is stirred for 5 minutes and 4 cc. of 15 N NaOH (0.06 moles) is added. The solution is stirred for 3 hours, and the solid product is separated by filtration and dried in a vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. The product is recrystallized from benzene, m.p. 67°C.

#### m-Methacrylamidobenzyl chloride

To 1.91 g. (0.01 M) of m-methacrylamidobenzyl alcohol 1.4 ml. of thionyl chloride is added. After 1 hour a solid appeared which was washed with water and then dilute sodium hydroxide and then water again. The solid was recrystallized from ethanol hexane

mixture m.p. 117°.

Attempted preparation of N-(3-methacrylamido) benzyl N,N-dimethyl-3-hydroxyanilinium chloride. Several attempts were made to prepare this salt by dissolving the corresponding chloride and tertiary amine in various solvents allowing the mixture to stand for a week. The solvents used were benzene ethanol, methanol and ether. In all cases a gummy polymer like mass was obtained.

m-Nitrodimethylaniline was prepared according to the method described in Organic Synthesis Coll. Vol. III, p. 658.

m-Aminodimethylaniline was obtained by reducing m-nitrodimethylaniline according to the method for the reduction of nitrobenzene with tin and HCl as described in Practical Organic Chemistry, A. I. Vogel, Longmans, Green and Co., London, 1957, p. 563.

m-Methacrylamidodimethylaniline was prepared by reacting equivalent amounts of methacrylic anhydride and m-aminodimethylaniline in cold water. The amide precipitates out and is recrystallized from ethyl acetate, m.p. 92°C.

m-Methacrylamidotrimethylanilinium iodide

A test tube containing 0.1 g. of m-methacrylamidodimethylaniline and 0.5 ml. methyl iodide is warmed in a water bath for 1 minute and then cooled in an ice bath. The tube is scratched and the product crystallizes out. The product is recrystallized from absolute ethanol, m.p. 191-192°C.

m-Acetamidotrimethylanilinium iodide is made by the same procedure used for the preparation of m-methacrylamidotrimethylanilinium

iodide only the amide was made with acetic anhydride instead of methacrylic anhydride, m.p. 202-203°C.

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iodide only the amide was made with acetic anhydride instead of methacrylic anhydride, m.p. 202-203°C.

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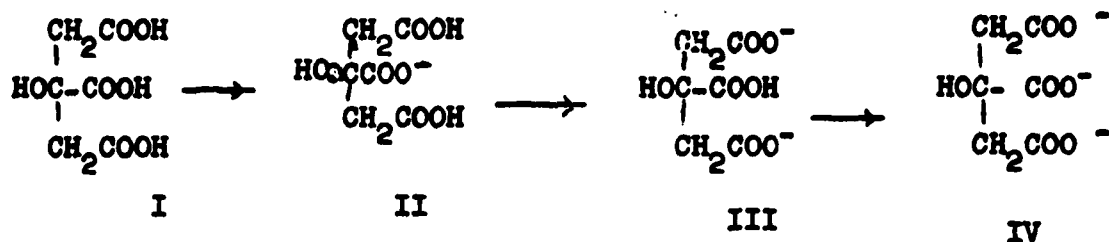
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B. The Carboxylate Catalyzed Hydrolysis of m-acetoxytrimethylanilinium Iodide

The second order rate constants for the hydrolysis of m-acetoxytrimethylanilinium iodide by various acids were obtained to determine whether the cationic ester would show an increase in susceptibility to carboxylate catalyzed hydrolysis when the charge on the catalyst is increased. No such effect is observed and the reactivity of the various ionic species varies more closely with its basicity than its charge.

It may be seen that with propane tricarboxylic acid and with succinic acid the reactivity of the carboxylate groups of the various ionic species increases with increasing basicity. However, a striking anomaly is observed with citric acid, for which the doubly ionized species is unexpectedly unreactive.

Because of the inductive effect of the hydroxyl and other electrostatic effects, the order of ionization of citric acid probably is



The anomalously low reactivity of species III may be interpreted in terms of hydrogen bonding. The carboxylates in species III can participate in hydrogen bonding with the hydroxyl group more easily than the carboxylate of species II. This intramolecular

hydrogen bonding reduces the nucleophilicity of species III. It would be desirable to determine whether the citric acid anomaly is also observed in the hydrolysis of an uncharged ester such as p-nitrophenylacetate.

**TABLE I**  
**CATALYSIS OF THE HYDROLYSIS OF A CATIONIC ESTER BY VARIOUS**  
**CARBOXYLATE GROUPS**

Acid	$10^6 k_A$ at 25°C	$k_A \times 10^6$ corrected for Statistical Factors	$10^5 k_2$ 1/M sec T=60.2°C	Reference for K <sub>n</sub>
Citric <sup>†</sup> <chem>CH2COOHCH(OH)CH2COOH</chem>	913 27.8 1.3	304 27.8 3.9	2.00 1.27 3.74	C. Morton Trans. Faraday Soc. 24, 22 (1928)
Propane <sup>†</sup> tricarboxylic <chem>CH2COOHCH(COOH)CH2COOH</chem>	32.5 26.5 1.48	108 26.5 4.44	2.00 4.84 8.99	
Glycolic <chem>HOCH2COOH</chem>	148	148	2.02	H. Harned and B. B. Owen, The Physical Chem. of Electrolytic Sol- utions, Rheingold N.Y. 1958, p. 755.
Acetic <chem>CH3COOH</chem>	17.5	17.5	4.64	
Succinic <chem>HOOCCH2COOH</chem>	63.8 2.69	31.9 5.38	3.2 6.3	H. S. Simms, J. Phys. Chem. 32, 1128 (1928)

<sup>†</sup>Determined at 30°C.

### Experimental

#### Materials

m-Acetoxydimethylaniline was prepared by acetylating m-hydroxydimethylaniline with acetic anhydride according to the method of Chattaway for acetylating phenols<sup>1</sup>, m.p. 38°, lit. m.p. 36.5°C.<sup>2</sup>

m-Acetoxytrimethylanilinium iodide was prepared by quaternizing the ester in acetone containing an excess of methyl iodide, m.p.

203°C. Anal. Calcd. for  $C_{11}H_{16}NO_2I$ : C, 41.12; H, 4.98; N, 4.36.

Found: C, 41.06, H, 4.95; N, 4.43.

m-Hydroxytrimethylanilinium iodide was prepared by reacting m-hydroxydimethylaniline with an excess of methyl iodide in methanol, m.p. 182°C, lit. m.p. 182°C.<sup>3</sup>

### Kinetics

Reaction rates were measured at 60.2°C. The second order rate constants for the various carboxylate groups were calculated from the slopes of plots of pseudo first order rate constants versus acid concentration.

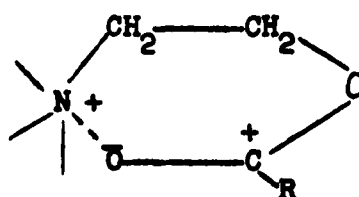
The rate of reaction was followed by observing the increase in optical density at 273 mμ ( $\epsilon = 2370$ ) associated with the production of m-hydroxydimethylanilinium iodide by means of a Beckman D.U. spectrophotometer. The concentration of ester used in all runs was  $2.1 \times 10^{-4}M$ .

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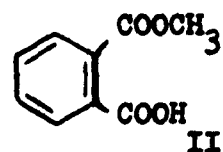
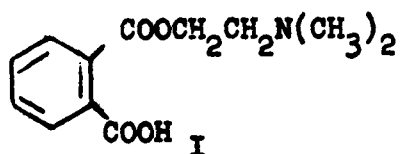
C. Hydrolysis of an Ester with a Neighboring Carboxyl and a Quaternary Ammonium Group

The enhanced reactivity of esters of the choline type has been explained<sup>1</sup> by assuming that the positively charged nitrogen stabilizes a negative charge on the carbonyl oxygen, making the carbonyl carbon more susceptible to attack by a nucleophilic reagent as shown below.



This type of catalysis has been classified by Bender<sup>2</sup> as intramolecular general acid catalysis. The difference in reactivity of esters of cyclohexanol derivatives with a cis or a trans quaternary nitrogen has been offered as evidence for the above mechanism.<sup>3</sup> By analogy, a neighboring positively charged nitrogen should make the carbonyl carbon more susceptible to neighboring carboxylate attack. It has been shown previously that an ester containing both an ionized and an unionized neighboring carboxyl groups is highly reactive, and this effect has been attributed to a stabilization of the transition state by hydrogen bonding between the carbonyl oxygen of the ester and the unionized carboxyl.<sup>4</sup> It was suggested that a similar stabilization might be achieved by ion-pair formation involving the partial negative charge of the carbonyl oxygen and a neighboring cationic group.

To demonstrate this effect, we carried out a study of the pH dependence of the rate of hydrolysis of  $\beta$ -N,N-dimethylaminoethyl hydrogen phthalate (I). An analogous ester lacking the cationic group, i.e., methyl hydrogen phthalate II has been investigated previously.<sup>5</sup>



The pseudo first order rate constants for the hydrolysis of ester I in various buffers and at different temperatures are given in Table I.

TABLE I  
PSEUDO FIRST ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF  
B,N,N DIMETHYLAMINO ETHYL HYDROGEN PHTHALATE

<u>pH</u>	<u><math>10^5 k \text{ sec}^{-1}</math></u> <u>(at 75°C)</u>	<u>pH</u>	<u><math>10^5 k \text{ sec}^{-1}</math></u>	<u>Temp. °C</u>
1.11	3.3	7.92	41.0	75.5
2.82	2.8	6.06	3.0	75.5
3.26	2.7	6.06	0.033	34.5
4.92	3.0	6.06	1.13	65.5
6.39	3.8	6.06	6.65	85.4
6.99	8.1			

In Figure 1 the logarithms of the rate constants obtained at 75.5°C are plotted against pH along with a similar plot for the hydrolysis of methyl hydrogen phthalate taken from reference (6).

An Arrhenius plot of rates obtained at pH 6.06 gave an activation enthalpy of 23.4 kcal/mole as against 33.7 kcal/mole reported for ester II<sup>6</sup>.

The results of this study show that the amphoteric ester I is much more reactive than the acid ester II, but that the

hydrolysis rate of I unlike that of II, is pH independent in the region of carbonyl ionization. It may therefore be concluded that in this pH region the bulk of the hydrolysis is due to direct attack of water on the ester group, and that any contribution which intramolecular carboxylate attack may make to the observed hydrolysis rate of I is within the experimental error of the rate constant measurements. If we extrapolate the data from reference 6 for the fully ionized II at 109° and 84° to a temperature of 75.5°, we find that intramolecular carboxylate attack on the ester group results in a hydrolysis rate constant of  $0.079 \times 10^{-5} \text{sec}^{-1}$ . This is less than 3% of the rate constant observed for ester I in the plateau region below pH 6.1, so that intramolecular carboxylate attack would not be experimentally detectable in ester I unless it were about four times as efficient as in II. Apparently the cationic group does not produce an effect of this magnitude.

#### EXPERIMENTAL

$\beta$ -N,N-dimethylaminoethyl hydrogen phthalate.— Phthalic anhydride (7.4 g, 0.05 mole) and 17.8 g (0.2 mole) dimethylaminoethanol were placed in a 250 ml three-neck round-bottom flask equipped with a mechanical stirrer. The mixture was stirred at

room temperature for six hours. The resulting slurry was washed with benzene and recrystallized from ethanol giving small colorless plates. Melts with decomposition 160-170°C.

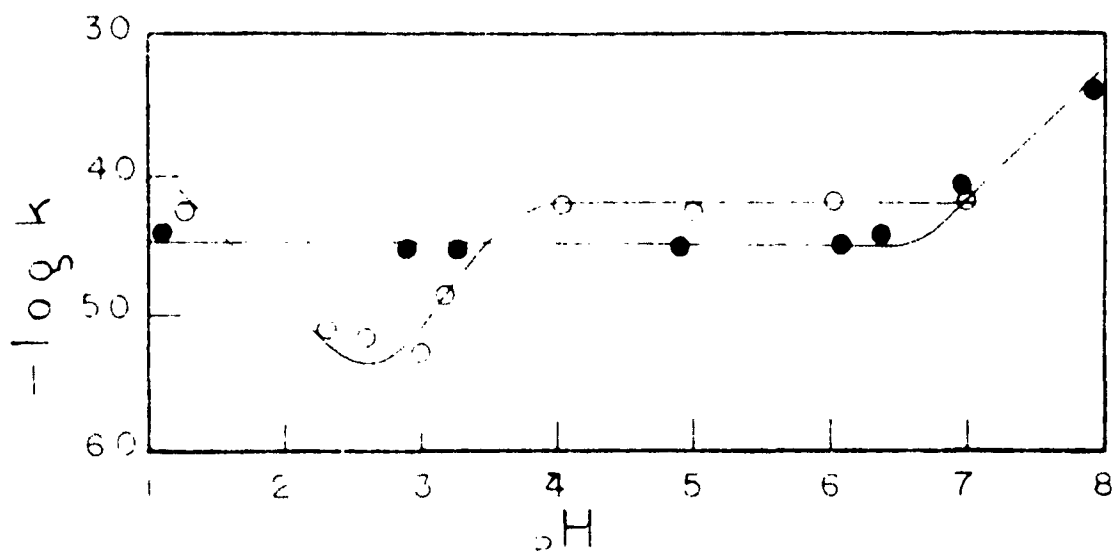
Anal. Calc. for  $C_{12}H_{15}NO_4$ : C, 60.75; H, 6.36; N, 5.90.

Found: C, 61.02; H, 6.46; N, 5.81.

Kinetics.- The hydrolysis reactions were carried out in buffer solutions less than 0.02 M in ionized acid. The ionic strength was adjusted to 0.1 M by addition of sodium chloride, and the pH was determined with a Cambridge Research Model pH meter. The rate of reaction was studied by observing the disappearance of the peak due to the ester at 280 mμ on a Beckman DU Spectrophotometer. In order to follow the rate of reaction in this manner the free phthalic acid had to be ionized, since unionized phthalic acid has approximately the same extinction coefficient as the ester. Therefore, for most runs 2 ml aliquots of the reaction solution (containing 24 mg. ester/100 ml) were transferred into 2 ml. of 0.5 M phosphate buffer (pH 6.06), and the optical density (D) determined at 25.3°C. Plots of  $-\ln(D-D_\infty)$  were linear in time in all cases, and were used to calculate the first order rate constant. The formation of phthalic acid in the reaction was proved by the similarity between the spectrum of the ester after hydrolysis and phthalic acid at the same pH.

Figure 1

The Hydrolysis of  $\beta$ -N,N-Dimethylaminoethyl  
Hydrogen Phthalate at 75.5°C, and of Methyl  
Hydrogen Phthalate at 109°, 0.<sup>5</sup>



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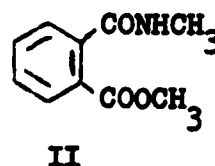
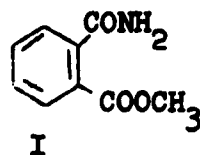
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D. The Effect of a Neighboring Amide Group on the Hydrolysis of Esters and Amides

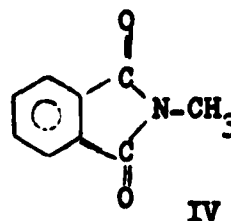
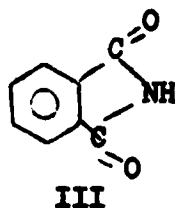
A. Introduction

While studying the equilibrium between acids, amines and amides, Paul Otaki of this laboratory observed the facile hydrolysis of amides with neighboring amide groups. The rapid hydrolysis of esters of aspartic acid residues in peptides had been reported by Bernhard.<sup>1</sup> At this time we began to study the hydrolysis of phthalamide, methylphthalamide and the methyl esters of phthamic acid and N-methylphthamic acid.

B. Hydrolysis of the Methyl Esters of Phthamic acid I and N-methylphthamic Acid II.

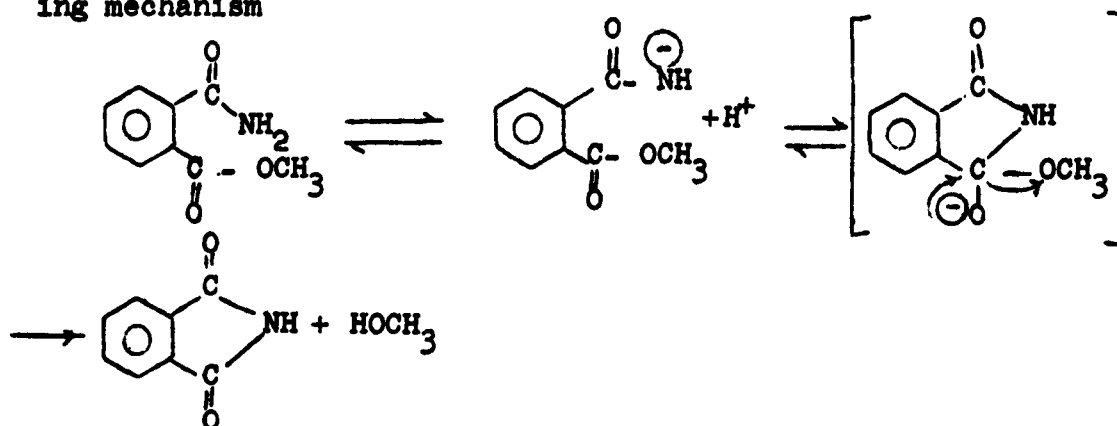


The hydrolysis of these esters were studied at pH 6-7.5. The products of the reactions were identified as the corresponding imides III and IV



by the congruence of the ultraviolet spectra of the products with those of imides III and IV.

The pseudo first order rate constants are tabulated below along with the corresponding activation energies. The pseudo first order rate constants were found to be proportional to the hydroxide ion concentration. This is in agreement with the following mechanism



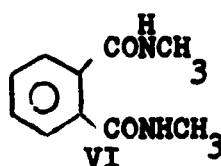
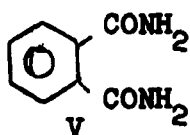
It was found that ester II was four times more reactive than ester I. Whether this is due to an increase in the nucleophilicity or a more favorable steric situation for the amide ion of species II is unresolved.

It would be desirable to study the effects of changing the acidity of the amide on its reactivity. This could be done by studying the hydrolysis of N-phenylmethylphthalamate and varying the p-substituents on the phenyl group. Such a study would have to be done in mixed solvent systems, since it was found that the N-phenylphthalimides are too water insoluble, even for the low concentrations involved in our method of analysis.

TABLE I  
Hydrolysis of the Imide and Methyl Ester of  
Phthalamic Acid and Methyl Phthalamic Acid

Compound	Ph at 26°C	$\mu=0.1$ Buffer System	T°C	kII l/mole sec	Ea kcal/ mole
phthalamide	8.78	0.024M $\text{H}_3\text{BO}_3$ + 0.01M $\text{NaOH}$	25.85	18.2	10.6
	8.78		33.98	36.4	
	8.78		42.13	54.9	
N-methylphthal- amide	8.78	↓	25.85	40.3	10.1
	8.78		33.98	61.6	
	8.78		42.13	95.9	
methylphthalamate	6.29	[ $\text{H}_2\text{PO}_4^-$ ] + [ $\text{HPO}_4^{2-}$ ] = 0.02M	25.85	3,220	10.3
	6.29		33.98	5,240	
	6.29		42.13	7,920	
N-methylmethyl- phthalamate	6.30	↓	25.85	12,800	10.3
	6.30		33.15	17,900	
	6.30		42.13	28,200	

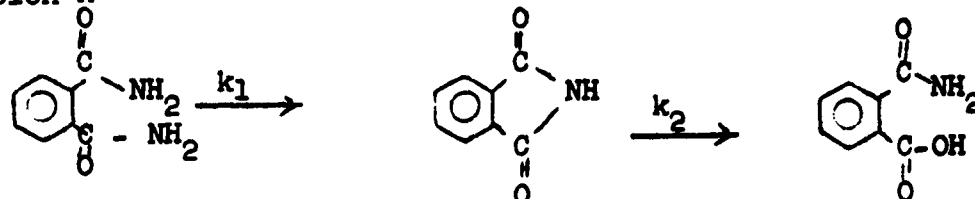
The Hydrolysis of Phthalamide V and Methylphthalamide VI



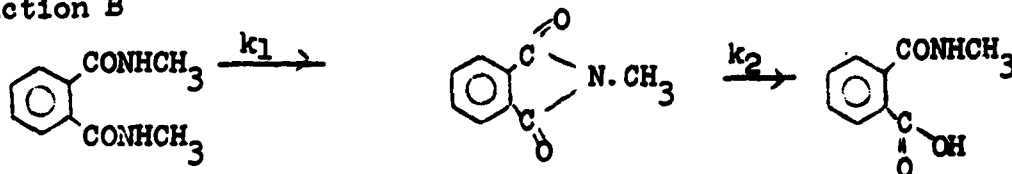
The reaction kinetics of V and VI were observed by following the appearance of the corresponding phthalimide at 299  $\mu$ . The kinetics were not first order, since the imide formed was more reactive than the amide. Thus the reaction followed the

following path

Reaction A



Reaction B



The final products were identified by the congruence of their ultra-violet spectra with that of phthalamic acid and N-methylphthalamic acid. By applying the equation for first order consecutive reactions to this system,  $k_1$  and  $k_2$  were obtained for both reactions. The  $k_2$  obtained separately from the hydrolysis of the imides agreed well with that obtained from the consecutive reaction, strongly indicating that the intermediates are the imides. The pertinent rate constants are tabulated below. The ratios of  $k_2/k_1$  obtained from the steady state imide concentrations were constant between 25°C and 45°C indicating that the activation energies for the first step are nearly equal to that for the hydrolysis of the imides, which are tabulated in in Table II.

TABLE II

Pseudo First Order Rate Constants for the Hydrolysis  
of Phthalamide and Methylphthalamide

$\mu = 0.1$        $T = 25.85$        $pH = 8.75 \pm 0.02$

		<u>Reaction A</u>
$10^4 k_1 \text{sec}^{-1}$	from time to reach steady state and	0.316
$10^4 k_2 \text{sec}^{-1}$	steady state imide concentration	1.31
$10^4 k_2 \text{sec}^{-1}$	from separate hydrolysis of imide	1.15
		<u>Reaction B</u>
$10^4 k_1 \text{sec}^{-1}$	from initial rate (below 1.5% completion	0.470
$10^4 k_2 \text{sec}^{-1}$	from separate hydrolysis of imide	2.55
$k_2/k_1$		5.43
$k_2/k_1$	calculated from state state imide concentration	5.52

At the completion of the work it was found that similar results had been obtained with the hydrolysis of succinamide which also implicated an imide intermediate.<sup>2</sup>

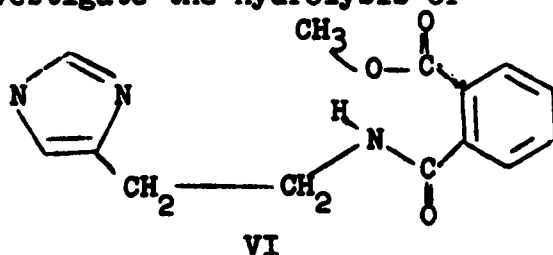
The hydrolysis of phthalimide seemsto have a positive salt effect. When the ionic strength is raised from 0.01 to 0.1 the second order rate constant increases from 12.05 l/mole sec (det at 25.35, pH=8.78) to 18.2 l/mol sec (det. at 25.35, pH=9.07).

The effect of weak bases on the hydrolysis of methylphthalamide is seen in Table III. Although there is a definite indication of basic catalysis, the effect of a weak base such

TABLE IIIEffect of Phosphate and Imidazole on the Trans-  
formation of Methylphthalamide to Methylphthalimide $\mu = 0.1$  $T = 25.85^\circ\text{C}$  $\text{pH} = 6.98$  $10^6 k_1 \text{sec}^{-1}$ Buffer

2.42	$[\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{=}]$	= 0.011M
2.78	$[\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{=}]$	= 0.02M
3.27	$[\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{=}]$	= 0.02M + 0.02M $\frac{1}{2}$ neutralized imidazole
3.21	0.02M $\frac{1}{2}$ neutralized imidazole	
3.37	0.04 M $\frac{1}{2}$ neutralized imidazole	
3.41	0.08M $\frac{1}{2}$ neutralized imidazole	

as imidazole seems to level off at higher concentrations. The effect of the base may be to help pull off a hydrogen from the amide nitrogen. If such is the case, it would be desirable to investigate the hydrolysis of



in which the basic nitrogen of the imidazole residue is held in the vicinity of the hydrogen on the amide nitrogen. The synthesis of compound VI has been undertaken.

Since enzymes have many amide bonds, further work on the

the neighboring amide group effect may help elucidate the mechanism of hydrolytic enzyme action.

### Experimental


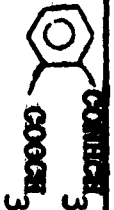
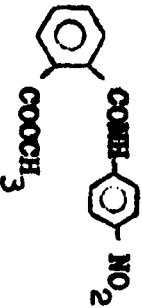
#### Materials

Phthalimide, methylphthalimide and phthalamide were obtained from Matheson and were recrystallized from acetone.

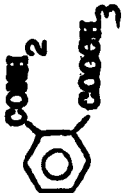

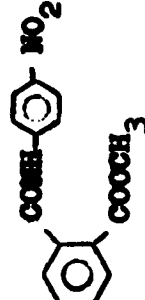
Methylphthalamide was prepared by the addition of phthaloyl chloride to an aqueous solution of methyl amine (20%). After a short time the amide precipitated out. It was recrystallized from acetone dec. 187°C reported dec. 185°C.<sup>3</sup>

The methyl esters of the N substituted phthalamic acids were prepared by reacting the silver salt of the corresponding acid with excess methyl iodide in dry acetone over night at room temperature, filtering off the silver iodide and recovering the ester by evaporating the acetone. The ester was recrystallized from benzene-hexane or acetone hexane mixture at room temperature. The silver salt of the acid was precipitated from an aqueous solution of the ammonium salt by the addition of a silver nitrate solution. The decomposition points of the esters depended on the rate of heating.

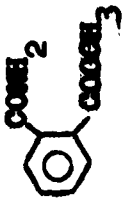

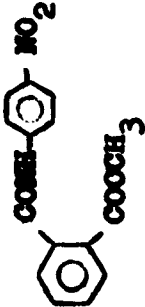
Identification of Reactants

<u>Ester</u>	<u>Empirical Formula</u>	<u>Calculated</u>			<u>Found</u>			<u>Dec. °C</u>
		<u>C</u>	<u>H</u>	<u>N</u>	<u>C</u>	<u>H</u>	<u>N</u>	
	$C_9H_9NO_3$	60.33	5.06	7.82	60.20	5.12	7.90	98-102°C (Ref. Dec.) 98-102°C
	$C_{10}H_{11}NO_3$	62.17	5.74	7.25	62.17	5.90	7.50	112-114
	$C_{15}H_{12}N_2O_5$	60.00	4.03	9.33	60.06	4.14	9.24	158-159

Identification of Reactants

<u>Ester</u>	<u>Empirical Formula</u>	<u>Calculated</u>				<u>Found</u>			<u>Dec. °C</u>
		<u>C</u>	<u>H</u>	<u>N</u>	<u>C</u>	<u>H</u>	<u>N</u>		
	$C_9H_9NO_3$	60.33	5.06	7.82	60.20	5.12	7.90		98-102°C (Ref. Dec.) 98-102°C
	$C_{10}H_{11}NO_3$	62.17	5.74	7.25	62.17	5.90	7.50		112-114
	$C_{15}H_{12}NO_5$	60.00	4.03	9.33	60.06	4.14	9.24		158-159

Identification of Reactants

<u>ester</u>	<u>Empirical Formula</u>	<u>Calculated</u>				<u>Found</u>			<u>Dec. °C</u>
		<u>C</u>	<u>H</u>	<u>N</u>	<u>C</u>	<u>H</u>	<u>N</u>		
	$C_9H_9NO_3$	60.33	5.06	7.82	60.20	5.12	7.90		98-102°C (Ref. Dec.) 98-102°C
	$C_{10}H_{11}NO_3$	62.17	5.74	7.25	62.17	5.90	7.50		112-114
	$C_{15}H_{12}NO_5$	60.00	4.03	9.33	60.06	4.14	9.24		158-159

### Kinetics

The reaction rates were measured with the aid of a Beckman ultraviolet spectrophotometer by observing the change in O.D. at 299 m $\mu$  which corresponds to the  $\lambda_{\text{max}}$  of the phthalimides. The reactions were carried out in water solution with supporting buffer and pH measured with a Cambridge Research Model pH meter.

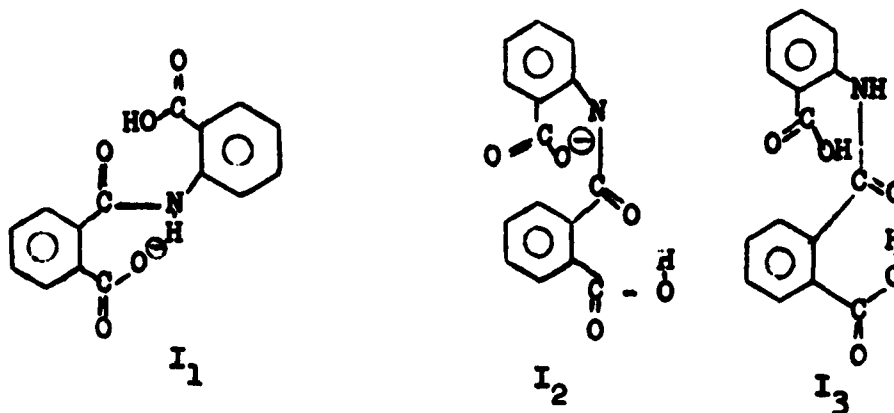
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## B. Hydrolysis of an Amide with two Neighboring Carboxyl Groups

### Introduction

The participation of a neighboring unionized carboxylic acid residue in the hydrolysis of an amide bond has been demonstrated in the case of phthalamic acid<sup>1,2</sup>. The proposed mechanism involves proton donation by the carboxyl group to the amide nitrogen or carbonyl oxygen, and attack on the carbonyl carbon by the resulting carboxylate to form phthalic anhydride and eliminate ammonia<sup>1,2</sup>. Considering the mechanism, it was hoped that further rate enhancement might be obtained by placing the amide bond between a carboxyl and a carboxylate group. The hydrolysis of N- [o-carboxy] phenylphthalamidic acid  $I_3$  was studied, therefore, with the expectation of obtaining a maximum in the pseudo first order rate constant at a pH corresponding to a maximum in species  $I_1$  or  $I_2$ .



### Results and Discussion

The following equation should predict the variation in pseudo first order rate constant with the degree of ionization  $\alpha_1$  and  $\alpha_2$  of the two carboxyl groups:

$$k_{\text{obs}} = k_1 \alpha_1 (1 - \alpha_2) + k_2 \alpha_2 (1 - \alpha_1) + k_3 (1 - \alpha_1)(1 - \alpha_2) + k_4 [H^+] \quad (1)$$

The first three terms on the right represent the contribution to the overall rate constant by each of species 1,2,3 respectively. The fourth term represents the contribution of acid catalysis to the overall rate constant. It is assumed that the contribution of the doubly ionized species to the hydrolysis rate is negligible in the pH range of this investigation. Equation 1 may be rewritten in the form

$$k = \frac{H[k_1 K_1 + k_2 K_2 + k_3] [H]^2 + k_4 [H]}{[(H^+) + K_1] [(H^+) + K_2]} \quad (2)$$

where  $K_1$  and  $K_2$  are the acid dissociation constants defined by

$$I_3 \xrightleftharpoons{K_2} I_2$$

$$I_2 \xrightleftharpoons{K_1} I_1$$

From the experimental results the five parameters of equation are calculated. They are tabulated in Table I along with the observed and predicted over-all rate constants.

TABLE I

Variation in Rate with Hydrogen Ion Concentration for the  
Hydrolysis of N-(o-carboxy)Phenylphthalamic Acid ( $I_3$ )

$$T = 25.80 \pm 0.05 \quad \mu = 0.1$$

$$k_{\text{obs}} = \frac{(k_1 K_1 + k_2 K_2)H + k_3 H^2}{(H + K_1)(H + K_2)} + k_4 H$$

$$k_1 K_1 + k_2 K_2 = 2.23 \times 10^{-6}$$

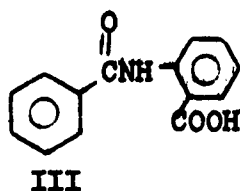
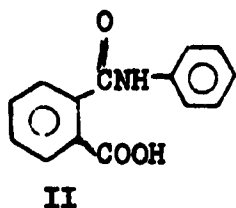
$$k_3 = 1.5 \times 10^{-4} \text{ sec}^{-1}, \quad k_4 = 18.1 \times 10^{-4} \text{ sec}^{-1}$$

$$K_1 = 5.5 \times 10^{-4} \quad K_2 = 1.8 \times 10^{-4}$$

$10^4 [H^+]$	$10^4 k_{\text{obs}}$ sec <sup>-1</sup>	$10^4 \text{ kcal.}$ sec <sup>-1</sup>
9,230	18.2	18.2
891	3.62	3.62
89.1	3.91	3.87
11.2	12.36	12.4
4.68	16.10	16.1
3.09	16.56	16.8
1.91	16.20	15.8
1.00	12.28	12.4
.251	4.81	4.8

The values of  $K_1$  and  $K_2$  calculated from the data in Table I are somewhat higher than one would expect by considering the values for phthalamic acid  $(1.6 \times 10^{-4})^3$  and benzoic acid  $(6.0 \times 10^{-5})^3$ . However, this discrepancy may be accounted for by recalling that ortho substituted benzoic acids increase in acidity with bulkiness of the ortho substituent.

To gain additional insight into the mechanism, the hydrolysis of N-benzoylanthranilic acid III and N-phenylphthalamic acid II were studied.



Preliminary studies have shown species III to be unreactive. From this, one may assume species  $I_1$  to be unreactive and  $k_1K_1$  to be negligible compared to  $k_2K_2$ . Using the values of  $(k_1K_1 + k_2K_2)$  and  $K_2$  calculated from the results in Table I, one may assign a value of  $0.0124 \text{ sec}^{-1}$  to  $k_2$ .

A comparison of  $k_2$  with  $k_3$  indicates that the catalytic efficiency of the neighboring carboxyl group is increased over eighty fold by the introduction of a neighboring carboxylate group. The variation in pseudo first order rate constant with pH for species II is given in Table II along with the expected values calculated from

$$k_{\text{over-all}} = k_4H + \frac{H}{H+K_1} k_3 \quad (3)$$

where  $K_1$  is the ionization constant of species II, and  $k_4$  and  $k_3$  are the contributions to the over-all rate constant by hydrogen ion and neighboring carboxyl catalysis.

The hydrolysis of phenylphthalamide is thus similar to that of phthalamide acid.

TABLE II

Variation in Rate with Hydrogen Ion Concentration for the  
Hydrolysis of N-Phenylphthalamic Acid (II)

$$k_{ov.} = k_4 H^+ + k_3 \frac{H^+}{H^+ + K_1}$$

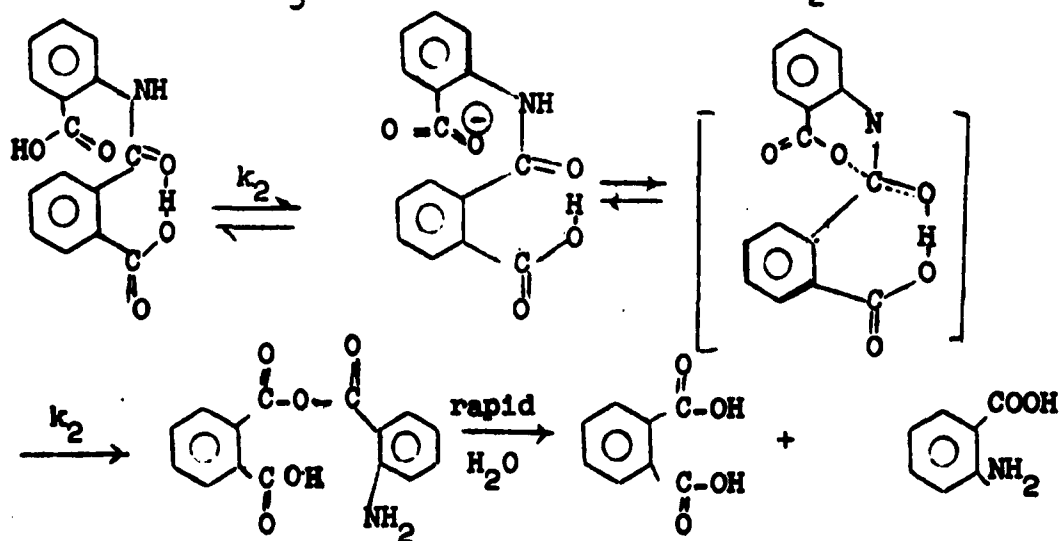
$$k_4 = 7.18 \times 10^{-4} \text{ l/mole/sec}$$

$$k_3 = 2.6 \times 10^{-4} \text{ sec}^{-1}$$

$$K_1 = 2.4 \times 10^{-4}$$

$10^4 H^+$	$10^4 k_{obs} \text{ sec}^{-1}$	$10^4 \text{ kcal/sec}^{-1}$
10,000	9.78	9.78
447	3.20	2.90
91.2	2.67	2.66
16.3	2.32	2.26
5.12	1.93	1.77
0.257	0.240	.251
0.163	0.170	.166

The following mechanism is proposed for portion of the hydrolysis of  $I_3$  which goes through species  $I_2$ .



The portion of  $I_3$  hydrolyzing through the unionized species probably follows the mechanism proposed for phthalamic acid.<sup>2</sup>

To further test the mechanism proposed for the hydrolysis of species  $I_3$ , the hydrolysis of N-[p-carboxy] phenylphthalamic acid is currently being investigated.

### Experimental

#### Materials

N-phenylphthalamic acid was prepared according to the method described in reference four. m.p. 169°C; lit. 169°C<sup>4</sup>. N-[o-carboxy]-phenylphthalamic acid was prepared in a similar manner. m.p. 175-6°C; lit. 172-3°C.<sup>5</sup>

N-benzoylanthranilic acid was prepared according to the method of reference six. m.p. 180-1°C, lit. 180-1<sup>6</sup>.

#### Kinetics

The rate hydrolysis of  $I_3$  was measured in aqueous buffer solution at 25.85°C by following the decrease in optical density at 305m $\mu$  or the increase in optical density at 350 m $\mu$ , corresponding to the decay of the amide or the production of anthranilic acid. Observations at both wavelengths gave identical first order rate constants. The products of each run were verified by the congruence between their ultraviolet spectra and that of a solution of anthranilic acid at the same pH and concentration as the product solution. The rate of hydrolysis of N-benzoyl-anthranilic acid was measured under

the same conditions as above except that the decrease of the amide concentration was observed by the change in optical density at 265 mμ.

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## F. The Effect of Polyacids on the Hydrolysis of a Cationic Ester

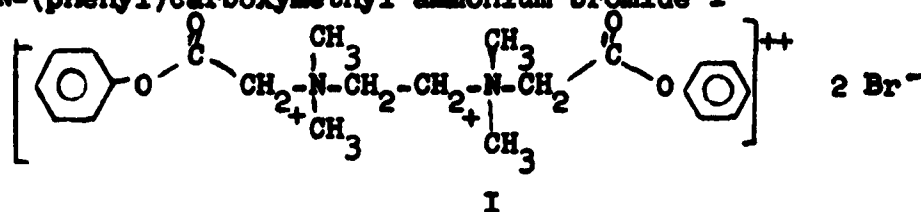
### I. Introduction

It has previously been found that the hydrolysis of cationic esters is inhibited by polyacids<sup>1</sup>. This phenomenon may be interpreted by molecular complexation of the polyanion with the cationic esters, so that the esters find themselves in a region from which hydroxyl ions are repelled by the large fixed negative charge on the polymer.

Morawetz has shown that this inhibition effect may be predicted from polyelectrolyte theory.<sup>2</sup>

It may also be shown that this effect is the mirror image of enzyme action. Previous investigations have considered only singly charged cationic esters. The effect of polyanions in these systems was small, and therefore, a theoretical treatment of the results was limited by possible experimental error.

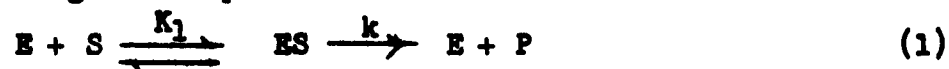
To investigate this phenomenon further the effect of polyanions on the hydrolysis rate of ethylenebis N,N-dimethyl N-(phenyl)carboxymethyl ammonium bromide I



A theory analogous to those used to interpret enzyme action as well as a treatment based on the general considerations of polyelectrolyte solutions were developed to explain the experimental results.

## II. The Effect of Polyanions on the Hydrolysis of I- Analogy with Enzyme Kinetics

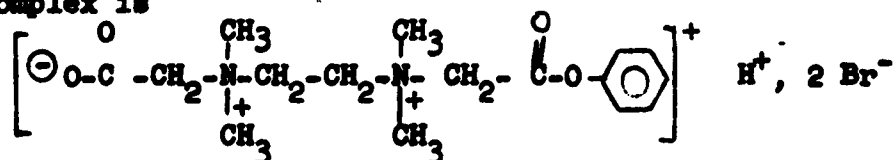
The kinetics of enzyme action may be represented by the following two step reaction



In the first step the enzyme (E) and the substrate (S) are in reversible equilibrium with the enzyme-substrate complex (ES). The highly reactive enzyme substrate complex then reacts to liberate free enzyme and a product which has a low affinity for the enzyme. Thus the enzyme is free to bind more substrate.

In our system the cationic ester becomes electrostatically bound to the polyanion. Unlike the enzyme-substrate system where small changes in the stereochemistry greatly alters the binding constant  $K_1$ , the binding between polymer and ester is probably electrostatic and nonspecific. The possibility of obtaining specific binding between a cation and a polyanion is the subject of a current investigation in this laboratory.

The ester in the polymer-ester complex is less reactive than the free ester, since the ester is held in a region of low concentration of the catalytic hydroxide ion. The hydroxide ions are repelled from the complex by the fixed negative charge on the polymer. The decomposition product of the polymer-ester complex is



II

Species II has a net positive charge of only one. It is bound to the polymer more weakly than the doubly charged ester I, and the polymer is less effective in inhibiting its hydrolysis.

Table I summarizes the differences between the enzyme-substrate system and the polymer ester system. The results leading to the conclusions in Table I are summarized in Tables II and I and Figure 1.

TABLE I

Comparison between Polymer-Ester System and  
Enzyme-Substrate System

<u>Characteristic</u>	<u>Enzyme-Substrate</u>	<u>Polymer-Ester</u>	<u>Supporting evidence for Polym.-Ester</u>	<u>Ref.</u>
Type of Binding	Specific	Non-specific electrostatic	Inhibition effect varies directly with fixed charge density on polymer and inversely with salt conc.	Table III Fig. I
Reactivity of Substrate in complex	Greater than free substrate	less than free substrate	addition of polymer causes inhibition	Table II and III
Affinity of product for Enzyme or polymer	low	low	polymer inhibits hydrolysis of species I more than species II	Table II

\* Fixed charge density varies with the degree of neutralization of the polyacid and the number of carboxylic acid residue per unit chain length.

FIGURE 1

The Effect of counterion concentration on the  
Efficiency of PMA as an inhibitor for the hydrolysis of

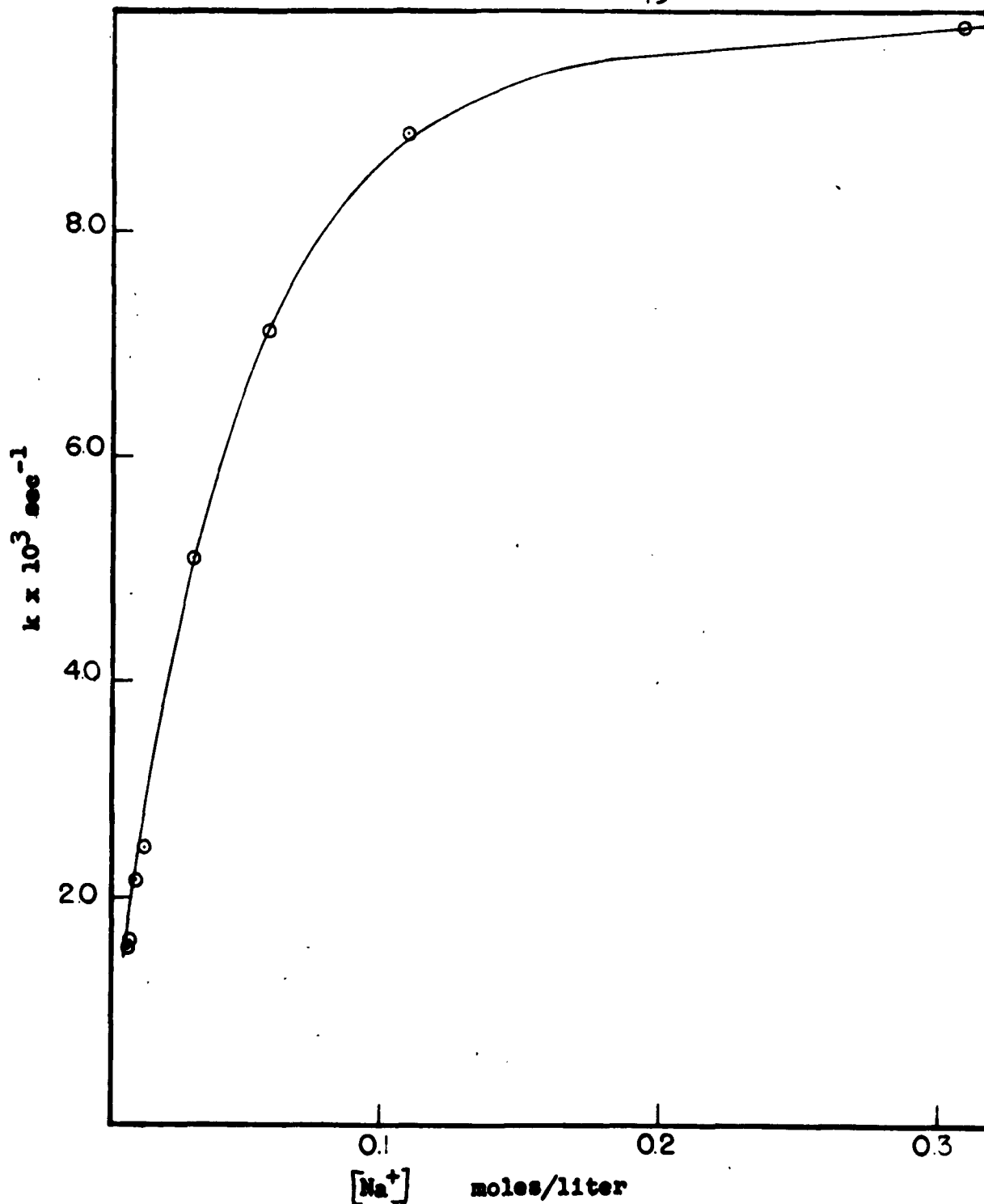
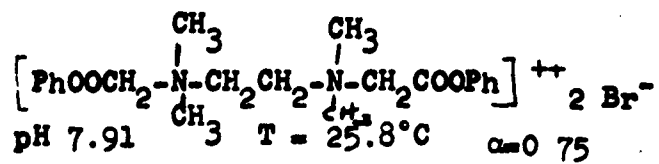
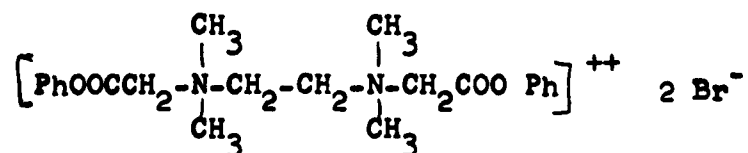


Figure 2

Analysis of the Polyanion Effect on the Hydroly-

sis of:

 $k$  = Rate constant with PAA/MA $\alpha \sim 0.66$  $k_0$  = Rate constant in absence of polymer

pH = 8.95      T = 2°C

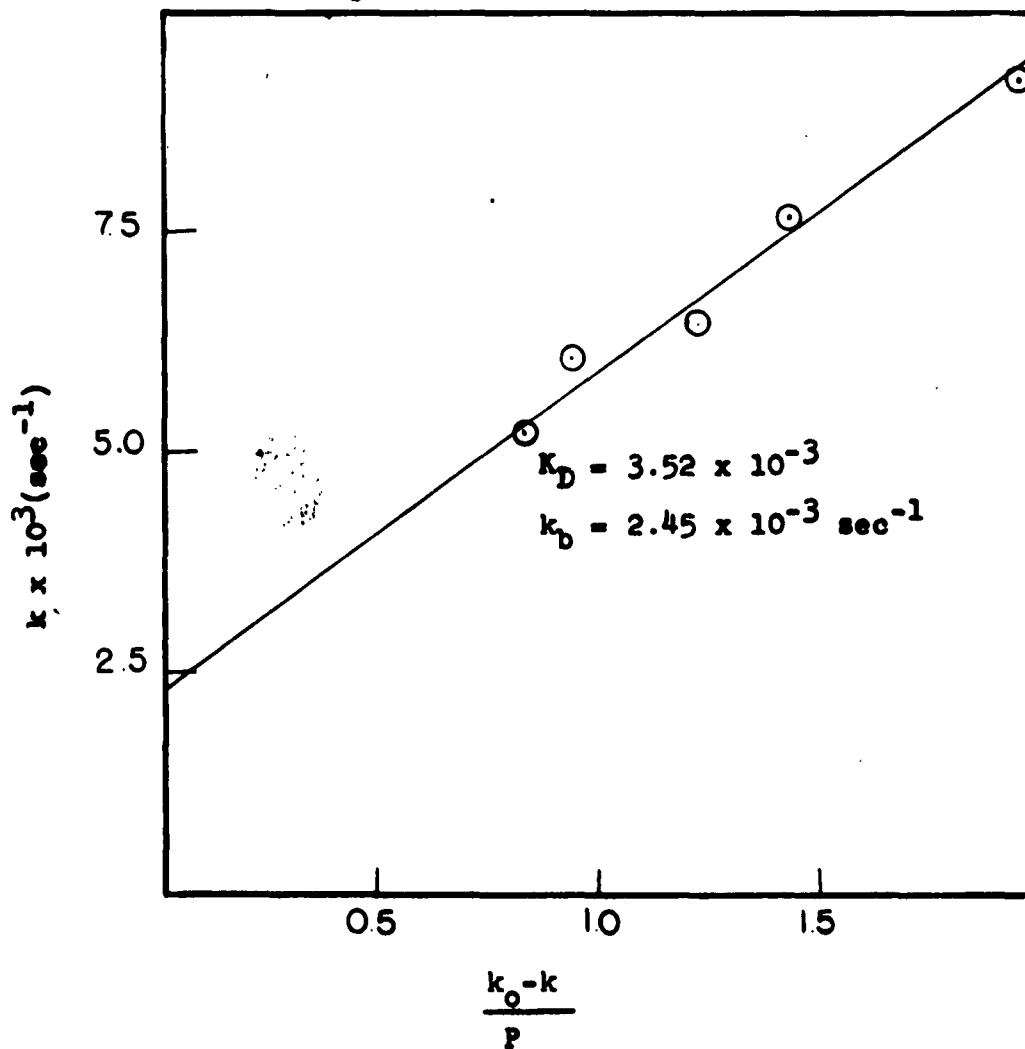
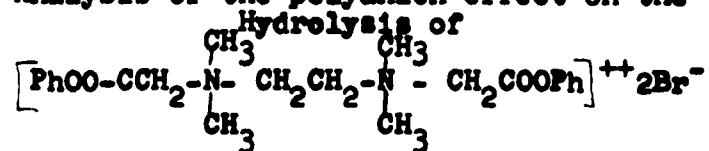
 $[\text{Na}^+] = 0.0367 \text{ M}$ 

Figure 3

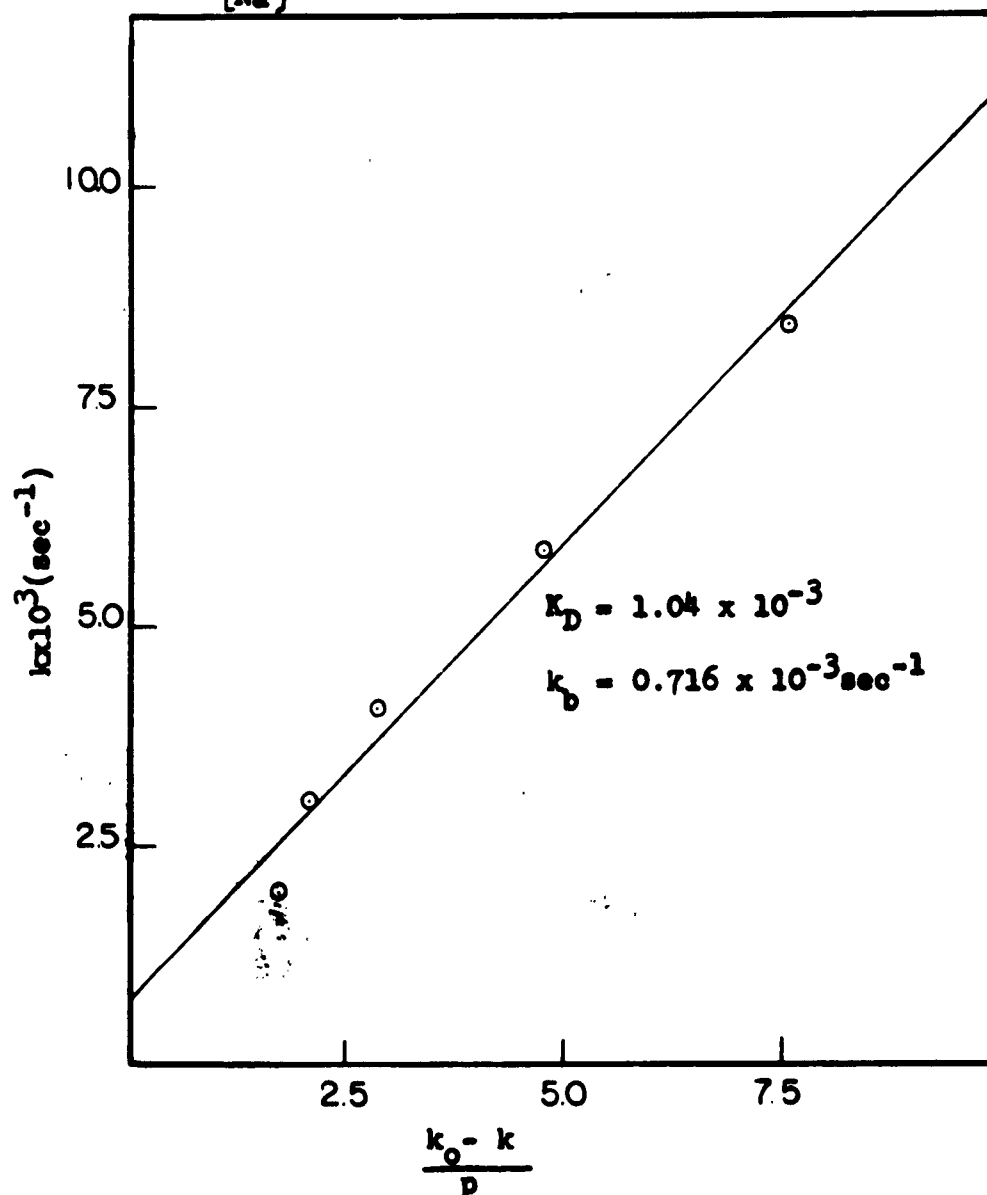
Analysis of the polyanion effect on the  
Hydrolysis of



pH = 8.97

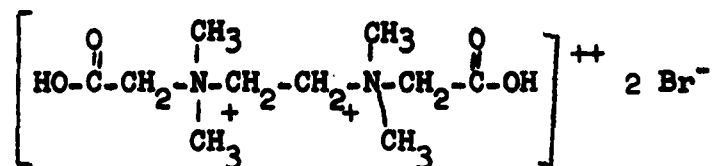
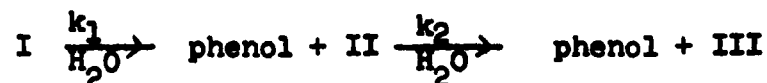
T = 2°C

$[\text{Na}^+] = 0.0184 \text{ M}$



**TABLE II**  
**Effect of 3/4 Neutralized Polymethacrylic Acid on the**  
**Hydrolysis of I and II**

1. Reaction Equation



III

2. Rate Equation

$$\frac{D_\infty + Dt}{D_\infty} = (1-\gamma) e^{-k_1 t} + e^{-k_2 t}$$

$$\gamma = \frac{k_1}{2(k_1 - k_2)}, \quad Dt = \text{optical density due to phenol}$$

$t$  = time in seconds

3. Results

<u>Conditions</u>	<u>pH</u>	<u><math>10^3 k_1 \text{sec}^{-1}</math></u>	<u><math>10^3 k_2 \text{sec}^{-1}</math></u>	<u>Temp. °C</u>
0.02M phosphate buffer	8.07	15.6	4.5	25.8
3/4 neut. 0.01 M polymethacrylic acid	8.02	2.1	1.6	25.8

TABLE IIIEffect of Polyanions on the Hydrolysis of I

T = 25.8°C			Polymer conc. 0.01 N		
Polymer	Degree of neut. $\alpha$	pH	$10^3 k_0 \text{ sec}^{-1}^*$	$10^3 k \text{ sec}^{-1}^{**}$	$k_0/k$
PMA	0.667	7.54	4.79	1.44	3.33
PAA/MA 0.0113N	0.591	7.88	10.2	0.865	11.8
PAA	0.667	7.42	3.63	0.91	3.99
PMA	0.750	8.02	14.5	2.08	6.98
PVME/MA	0.750	9.05	151	6.68	22.6
PAA/MA 0.0113N	0.664	8.67	63.1	2.3	27.4
PMA	0.333	6.62	1.02	0.61	1.67
PMA 0.1M	0.667	6.83	1.38	1.39	1.01

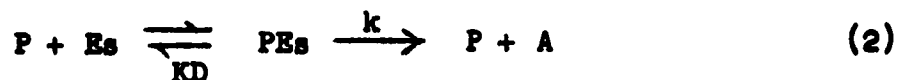
SymbolMeaning

PAA	polyacrylic acid
PMA	polymethacrylic acid
PAA/MA	polyacrylic acid-maleic acid 1-1 copolymer
PVME/MA	polyvinylmethylether-maleic acid copolymer

\*  $k_0$  rate constant for hydrolysis of I in presence of buffer

\*\*  $k$  rate constant for hydrolysis of I in presence of polymer

Although the effects in our system may be considered a mirror image of enzyme action, equation (1) may be developed for the polymer-ester system. Equation (1) may be rewritten as



P = polymer  
Es = I  
A = II

$$K_D = \frac{[P][E_s]}{[PEs]} \quad (3)$$

$$k = K_D + \frac{[PEs]}{[PEs] + [E_s]} (k_b - k_o) \quad (4)$$

$k$  = observed rate constant for hydrolysis of I in presence of polymer

$k_o$  = observed rate constant for hydrolysis of I in presence of buffer

$k_b$  = rate constant for hydrolysis of I in the polymer ester complex

combining (3) and (4)

$$k = k_b + K_D \frac{k_o - k}{P} \quad (5)$$

Equation (5) suggests a linear relationship between  $k$ , and a function of the measurable quantities  $k_b, k_o$  and  $[P]$ . However, to test equation (5) one must keep  $K_D$  and  $k_b$  constant. To accomplish this the counterion concentration should be kept constant and large in comparison to the polymer concentration. The pH should also be kept constant.

Equation (5) was tested with 3/4 neutralized copolymer PAA/MA at two different salt concentrations (Figures 2 and 3). The results fit equation (5) well if the salt concentration is more than twice the polymer concentration. As one would expect for electrostatic binding, the dissociation constant increases with counterion concentration (counterions compete with the ester for sites on the polyanion). Less meaningful results were obtained when an attempt was made to test equation (5) for 3/4 neutralized PMA. When the counterion

concentration was adjusted to more than twice the polymer concentration  $k_0$ - $k$  became small and subject to large error.

### III. The Application of Polyelectrolyte Solution Theory to the Polymer-Ester System.

Since the inhibition of the hydrolysis of I by polymer seemed to arise from electrostatic interactions it was thought that polyelectrolyte solution theory could be developed to account for this effect. Such a development is shown below.

The rate of ester hydrolysis is given by

$$V_H = k \langle OH^- \rangle \langle Es^{++} \rangle \quad (6)$$

where  $k$  is the observed rate constant, and  $\langle OH^- \rangle$  and  $\langle ES \rangle$  are the bulk average concentrations of hydroxide ion and ester (I), respectively. The velocity of the reaction may also be written as

$$V_H = k_0 \langle OH^- \times Es^{++} \rangle \quad (7)$$

where  $k_0$  is the intrinsic rate constant for the hydrolysis of the ester ( $k_0$  corresponds to the rate constant observed in buffer) and  $\langle OH^- \times Es^{++} \rangle$  is the product of hydroxide ion and ester concentration averaged over all volume elements.

Equating (6) and (7) and rearranging

$$\frac{k_0}{k} = \frac{\langle OH^- \rangle \langle Es^{++} \rangle}{\langle OH^- \times Es^{++} \rangle} \quad (8)$$

From electrostatic theory

$$\frac{[OH^-]}{[OH^-]_0} = e^{-\epsilon\psi/kT} = x \quad (9)$$

where  $[OH^-]$  is the concentration of hydroxyl ions,  $e$  is the electronic charge,  $\psi$  the electrostatic potential in a volume element, and  $[OH^-]_0$  is the hydroxide ion concentration in a volume element of zero electrostatic potential. Also, for the singly and doubly ionized positively charged species

$$\frac{[Es^{++}]}{[Es^{++}]_0} = \frac{1}{x^2} \quad \frac{[Na^+]}{[Na^+]_0} = \frac{1}{x} \quad (10)$$

substituting (10) into (8) we obtain

$$\frac{k_0}{k} = \frac{\langle x^{-2} \rangle \langle x \rangle}{\langle x^{-1} \rangle} \quad (11)$$

for the inhibition factor. It may be seen that the inhibition factor depends on the distribution of the electrostatic potential in the polyelectrolyte solution. As suggested by Morawetz<sup>2</sup>, if one carries out experiments with reagents of various charge types, one may obtain a number of different volume averages of the electrostatic potential to characterize the distribution of the electrostatic potential in the polymer solution.

To simplify the situation for our case we will assume only two distinct regions, one containing the polymer and the other from which polymer is excluded. This is equivalent to postulating that the electrostatic potential is a step function with only two values. The use of the Donnan theory of polyelectrolyte solutions will now be profitable. A pictorial

representation of this theory is shown in Figure 4. From the assumption of electroneutrality inside and outside the polymer region one obtains

$$S = S_0 (1-\phi) + \phi S_0 X_1 \quad (12)$$

$$S_0 = S / (1-\phi) + \phi X_1$$

where

$S$  = bulk average salt concentration

$S_0$  = salt concentration outside polymer region

$$X_1 = \frac{[\text{OH}^-]_{\text{inside}}}{[\text{OH}^-]_0}, \quad X_0 = 1$$

$\phi$  = volume fraction occupied by polymer

From the requirement of equal chemical potential of the mobile ions inside and outside the polymer regions one obtains

$$S_0^2 = S X_1 (S X_1 + p) \quad (13)$$

where  $p = P/\phi$  is the concentration charges within the polymer region. As a first approximation

$$S = S_0$$

Thus  $S X_1^2 + p X_1 - S = 0$

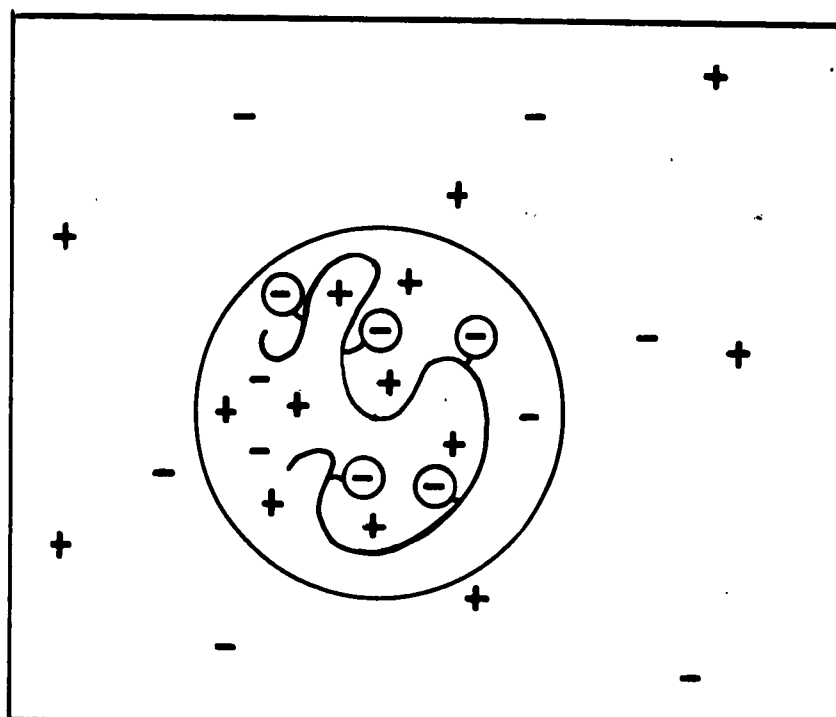
$$\text{and } X = \frac{-p + \sqrt{p^2 + 4S^2}}{2S} = \frac{-P}{2S\phi} + \sqrt{\frac{P^2}{4S^2\phi^2} + 1} \quad (14)$$

from equation 11

$$\frac{k_0}{k} = \frac{\langle X^{-2} \rangle \langle X \rangle}{\langle X^{-1} \rangle} = \frac{(\phi X_1^{-2} + 1 - \phi)(\phi X_1 + 1 - \phi)}{\phi X_1^{-1} + 1 - \phi} \quad (15)$$

Figure 4

# DONNAN MODEL



## Conditions

(1) Electroneutrality:  $p + S_i^- = S_i^+$   
 $S_o^- = S_o^+$

(2) Equal chemical potential:

$$S_i^- S_i^+ \gamma_i^2 = S_o^- S_o^+ \gamma_o^2$$

Equations 14 and 15 may be used to predict the inhibition factor in terms of only one adjustable parameter  $p$  ( $\phi = P/p$ ). Equation (15) was tested by conducting experiments at constant fixed charge density. This was accomplished by holding the counterion concentration constant. From figure 5 it may be seen that equation (15) predicts a value of  $p$  between 0.2 and 0.3 for counterion concentrations of 0.0367 and 0.0184M. In the calculation of  $k_o/k$  the deviation of  $S$  from  $S_o$  was taken into account by using eq. (12) in a second approximation.

Straus and Ander<sup>3</sup> have pointed out that the thickness of the counter-ion atmosphere around highly charged polyion is small compared to the radius of curvature of the polyion. We may therefore picture the region occupied by the polyion and its counter-ions as cylindrical. Knowing the fixed charge density on the polyion and the parameter  $p$ , we may calculate  $r$ , the radius of the cylindrical region assigned to the polyelectrolyte. In this manner we obtained

$$p = 0.2 \quad r = 34.5 \text{ \AA}^{\circ}$$

$$p = 0.3 \quad r = 28.1 \text{ \AA}^{\circ}$$

According to Straus and Ander<sup>3</sup> the radius of a polyelectrolyte rod-like molecule may be estimated from the value of the Debye shielding length for a 1-1 electrolyte multiplied by a factor  $S$ , which is close to one,

For a counter-ion concentration of 0.0367 M at 2°C  
 $1/K = 16.0 \text{ \AA}^{\circ}$ .

(values for  $1/K$  calculated from H. Harned and B.B. Szwed, The Physical Chem. of Electrolytic Solutions, 3rd Ed., Reinhold, N.Y., 1958, p. 68 eq. 3-1-5)

Figure 5

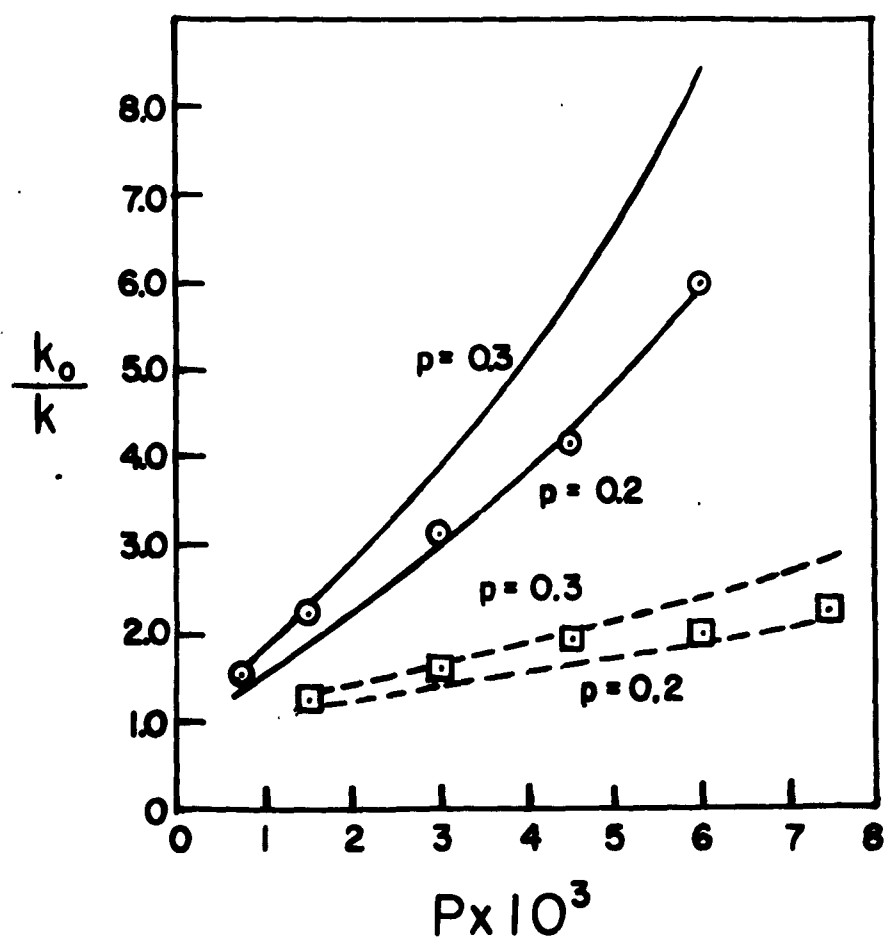
# Calculation of Inhibition Factor From Donnan Theory of Polyelectrolyte Solutions

Polymer PAA/MA,  $\alpha \sim 0.66$ , pH=8.97

Temp. 2°C

----  $\square$   $S = 0.0367$

—  $\circ$   $S = 0.0184$



For a counterion concentration of 0.0184 at 2°C

$$1/K = 22.6 \cdot A^\circ$$

These values agree well with the values of  $r$  predicted from the value of  $p$ . The factor  $S$  needed to make  $1/K$  equal to is close to the value found by Strauss and Ander<sup>3</sup>.

In conclusion it may be said that our results agree with the assumptions involved in the Donnan theory of poly-electrolyte solutions. Not only are the results represented by a theory involving a single adjustable parameter, but the value of this parameter agrees with the value predicted from the Debye-Hückel theory.

### Experimental

#### Materials:

Phenyl 2-bromoethanoate was prepared by reacting phenol with bromoacetyl bromide until all the hydrogen bromide was boiled off. The product was vacuum distilled from the mixture, b.p. 80°C at 2 mm. On cooling, the product solidified m.p. 31-32. The product was then recrystallized from benzene-hexane, m.p. 32°C, reported m.p. 32°C.<sup>4</sup>

Ethylenebis N,N-dimethyl N-(phenyl) carboxymethyl-ammonium bromide was prepared by reacting an excess of phenyl-2-bromoethanoate with tetramethylethylenediamine in dry nitromethane at room temperature, dec. 186°C.

Anal. Calc. for  $C_{22}H_{30}Br_2N_2O_4$ : C, 48.36; H, 5.53; Br, 29.26; N, 5.12. Found: C, 48.35; H, 5.62; Br, 29.28, N, 5.08.

Polymethacrylic acid was made by polymerizing 120 ml of methacrylic acid in 550 ml of methylethyl ketone containing 0.35 g. of azobisisobutyronitrile. The reaction was carried out under nitrogen in a large round bottomed flask. The polymer was washed repeatedly with ether.

Polyvinylmethylether maleic acid copolymer was obtained by hydrolyzing in water at 50°C for 12 hrs. a polyvinylmethylether maleic anhydride copolymer obtained from General Aniline and Film Corporation.

Polyacrylic acid maleic anhydride 1-1 copolymer was obtained by polymerizing 21.6 ml of acrylic acid with 120 g of maleic anhydride in 200 ml of methylethylketone containing 50mg of azobisisobutyronitrile for 48 hours at 60°C. The polymerization was carried out under nitrogen. The polymer was precipitated from the methylethyl ketone by slowly adding the solution to an excess of anhydrous ether.

Polyacrylic acid was prepared by heating at 60°C for 2 hours 100 ml of acrylic acid in 700 ml of benzene containing 500 mg of azobisisobutyronitrile. The reaction was carried out under nitrogen. The resulting polymer was repeatedly washed with ether.

### Kinetics

The rate of hydrolysis of the cationic esters I and II was followed by observing the change in optical density at 273 mμ (corresponding to the appearance of phenol) with the

aid of a Beckman D.U. spectrophotometer. Since  $k_1$  and  $k_2$  could not be found independently, three methods of analysis were devised to obtain  $k_1$  and  $k_2$  from the rate equation and the experimental results.

Method (1) -  $k_1$  and  $k_2$  from Initial and Final Slopes

The rate equation

$$\frac{D_\infty - D_t}{D_\infty} = (1-\gamma)e^{-k_1 t} + \gamma e^{-k_2 t} = y$$

gives for the initial slope  $t \rightarrow 0$   $y \sim 1$

$$\frac{d \ln y}{dt} = \frac{-k_1}{2}$$

for the final slope

$$t \rightarrow \infty \quad y \rightarrow 0$$

$$k_1 > k_2$$

$$\frac{d \ln y}{dt} = -k_2$$

$$k_1 < k_2$$

$$\frac{d \ln y}{dt} = -k_1$$

Method (1) is fast and fairly accurate for initial slopes, but subject to error in the determination of final slopes, since small errors in  $D_\infty$  will cause large errors in the determination of the final slope.

Method (2) - Curve fitting

This method involved plotting curves of  $\ln y$  versus  $k_2 t$  on log paper for several values of  $\gamma$ . From the experimental data a plot of  $\ln y$  versus time ( $t$ ) was then made. This plot was

overlayed on the calculated plot and moved horizontally until it fit over one of the calculated curves. The value of  $\gamma$  was thus obtained. The value of  $k_2$  was obtained by dividing any abscissa value on the theoretical curve by the corresponding value on the experimental curve. The value of  $k_1$  was calculated from  $k_2$  and  $\gamma$ .

This method is accurate, but time consuming, and only worthwhile if one has to calculate many rate constants with similar values of  $\gamma$ .

#### Method (3) - Modified curve fitting

From the rate equation, curves of  $k_2 t$  versus  $\gamma$  for various values of  $\ln y$  (0.1, 0.2, 0.3...1.6) were made. A plot of  $-\ln y$  versus time was made from the experimental data. Values of  $-\ln y$  on the experimental curve were chosen to correspond to the values of  $-\ln y$  for which curves were calculated. These values were tabulated along with the corresponding times. The times were layed off as distances on a straight line with the use of the log scale on the semi log paper. The straight line was held parallel to the ordinate and overlayed on the calculated plot, so that each point on the overlay was on the theoretical curve of corresponding  $\ln y$ . The point at which the straight line cut the x-axis gave  $\gamma$ , and an ordinate of a theoretical curve divided by the corresponding value of the time on the overlay gives  $k_2$ .  $k_1$  was then obtained from  $\gamma$  and  $k_2$ . This method avoids the interpolation between curves (values of  $\gamma$ ) involved in method (2).

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2. H. Morawetz, J. Polymer Sci., 42, 125 (1960).
3. U. Straus and P. Ander, J. Am. Chem. Soc., 80, 6494 (1958).
4. Kunckell and Scheven, Ber., 31, 172 (1898).

### PUBLICATIONS

Section "C" of "Results and Discussions" is identical with a paper accepted for publication by the Journal of Organic Chemistry.

Papers incorporating the results summarized in the other sections are being prepared and will be submitted to appropriate journals in due course.

### IDENTIFICATION OF PERSONNEL

Herbert Morawetz, the supervisor of the project, has been on the faculty of the Polytechnic Institute of Brooklyn since 1951. He was assistant professor in 1951-53, associate professor 1953-58 and professor of polymer chemistry since 1958. He is the author or co-author of about 50 scientific papers in the field of physical chemistry of high polymers.

Jules Shafer carried out as senior research fellow all the experimental work described in this report. He has a B.S. degree from City College of New York and is expected to complete the requirements for a Ph.D. degree some time in 1962.

Fabio Bruschtein was employed on the project from Nov. 1, 1960 to Aug. 31, 1961 as a senior research fellow. His work on the interaction of optically active polymeric acids with optically active bases was inconclusive and is not included in this report.